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=> d his

     (FILE 'HOME' ENTERED AT 09:46:39 ON 18 MAR 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 09:47:21 ON 18 MAR 2004
L1        354831 S BACILLUS
L2           107 S "TTGACA" AND "TATAAT"
L3            33 S L1 AND L2
L4         49123 S "AMYQ" OR "ALPHA-AMYLASE"
L5             0 S L1 (A)EMYLOLIQUEFACIENS
L6          5130 S L1 (A)AMYLOLIQUEFACIENS
L7             4 S L2 AND L6
L8             4 DUP REM L7 (0 DUPLICATES REMOVED)
L9            10 S " MRNA" AND L3
L10            6 DUP REM L9 (4 DUPLICATES REMOVED)
L11          788 S "CRYIIIA" OR "SP82"
L12            4 S L3 AND L11
L13            3 DUP REM L12 (1 DUPLICATE REMOVED)
                 E WIDNER W/AU
L14          110 S E3-E8
                 E SLOMA A/AU
L15          193 S E3-E7
                 E THOMAS M D/AU
L16          387 S E3
L17          652 S L14 OR L15 OR L16
L18            4 S L3 AND L17
L19            3 DUP REM L18 (1 DUPLICATE REMOVED)
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 NEWS  2          "Ask CAS" for self-help around the clock
 NEWS  3  SEP 09  CA/CAplus records now contain indexing from 1907 to the
                  present
 NEWS  4  DEC 08  INPADOC: Legal Status data reloaded
 NEWS  5  SEP 29  DISSABS now available on STN
 NEWS  6  OCT 10  PCTFULL: Two new display fields added
 NEWS  7  OCT 21  BIOSIS file reloaded and enhanced
 NEWS  8  OCT 28  BIOSIS file segment of TOXCENTER reloaded and enhanced
 NEWS  9  NOV 24  MSDS-CCOHS file reloaded
 NEWS 10  DEC 08  CABA reloaded with left truncation
 NEWS 11  DEC 08  IMS file names changed
 NEWS 12  DEC 09  Experimental property data collected by CAS now available
                  in REGISTRY
 NEWS 13  DEC 09  STN Entry Date available for display in REGISTRY and CA/CAplus
 NEWS 14  DEC 17  DGENE: Two new display fields added
 NEWS 15  DEC 18  BIOTECHNO no longer updated
 NEWS 16  DEC 19  CROPU no longer updated; subscriber discount no longer
                  available
 NEWS 17  DEC 22  Additional INPI reactions and pre-1907 documents added to CAS
                  databases
 NEWS 18  DEC 22  IFIPAT/IFIUDB/IFICDB reloaded with new data and search fields
 NEWS 19  DEC 22  ABI-INFORM now available on STN
 NEWS 20  JAN 27  Source of Registration (SR) information in REGISTRY updated
                  and searchable
 NEWS 21  JAN 27  A new search aid, the Company Name Thesaurus, available in
                  CA/CAplus
 NEWS 22  FEB 05  German (DE) application and patent publication number format
                  changes
 NEWS 23  MAR 03  MEDLINE and LMEDLINE reloaded
 NEWS 24  MAR 03  MEDLINE file segment of TOXCENTER reloaded
 NEWS 25  MAR 03  FRANCEPAT now available on STN

 NEWS EXPRESS  MARCH 5 CURRENT WINDOWS VERSION IS V7.00A, CURRENT
               MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP),
               AND CURRENT DISCOVER FILE IS DATED 3 MARCH 2004
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                                                           ENTRY    SESSION
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FILE 'LIFESCI' ENTERED AT 09:47:21 ON 18 MAR 2004
COPYRIGHT (C) 2004 Cambridge Scientific Abstracts (CSA)


=> s bacillus
L1      354831 BACILLUS

=> s "TTGACA" and "TATAAT"
L2         107 "TTGACA" AND "TATAAT"

=> s l1 and l2
L3          33 L1 AND L2

=> s "amyQ" or "alpha-amylase"
L4       49123 "AMYQ" OR "ALPHA-AMYLASE"

=> s l1 (a)emyloliquefaciens
L5           0 L1 (A) EMYLOLIQUEFACIENS

=> s l1 (a)amyloliquefaciens
L6        5130 L1 (A) AMYLOLIQUEFACIENS

=> s l2 and l6
L7           4 L2 AND L6

=> dup rem l7
PROCESSING COMPLETED FOR L7
L8           4 DUP REM L7 (0 DUPLICATES REMOVED)
```

=> d 1-4 ibib ab

L8 ANSWER 1 OF 4 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a Bacillus cell in a medium conducive to the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: WIDNER W; SLOMA A; THOMAS M D
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003
APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]
AB DERWENT ABSTRACT:

NOVELTY - Producing a polypeptide comprises cultivating a Bacillus cell in a medium conducive to the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a Bacillus cell comprising a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a Bacillus host cell by introducing into a Bacillus cell the nucleic acid construct cited above; (3) a method for producing a selectable marker-free mutant of a Bacillus cell by deleting a selectable marker gene of the Bacillus cell; and (4) a selectable marker-free mutant of a Bacillus cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter comprises two or more bacterial promoter sequences, which are obtained from one or more Bacillus genes. The tandem promoter comprises the amyQ promoter, a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** from the -10 region, the amyL promoter, and/or the cryIIIA promoter. The tandem promoter comprises two copies of the amyQ, amyl or cryIIIA promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the Bacillus cell. The mRNA processing/stabilizing sequence is the cryIIIA or SP82 mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The Bacillus cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the Bacillus cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase,

glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the Bacillus cell. The Bacillus host cell is Bacillus alkalophilus, **Bacillus amyloliquefaciens**, **Bacillus** brevis, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus coagulans, Bacillus firmus, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus sterothermophilus, Bacillus subtilis, or Bacillus thuringiensis. This method alternatively comprises cultivating a Bacillus cell in a medium conducive for the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a Bacillus promoter. Preferred Cell: The Bacillus cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a Bacillus cell, and for producing a selectable marker-free mutant of a Bacillus cell.

EXAMPLE - No relevant example given.(57 pages)

L8 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER:	2003:913313 HCAPLUS
DOCUMENT NUMBER:	139:376224
TITLE:	Homologous recombination into Gram-positive bacterium for generation of expression libraries of polynucleotides
INVENTOR(S):	Bjornvad, Mads Eskelund; Jorgensen, Per Lina; Hansen, Peter Kamp
PATENT ASSIGNEE(S):	Novozymes A/S, Den.
SOURCE:	PCT Int. Appl., 55 pp. CODEN: PIXXD2
DOCUMENT TYPE:	Patent
LANGUAGE:	English
FAMILY ACC. NUM. COUNT:	1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003095658	A1	20031120	WO 2003-DK301	20030507

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: DK 2002-682 A 20020507

AB A method for generating an expression library of polynucleotides, wherein each polynucleotide is integrated by homologous recombination into the genome of a competent Gram-pos. bacterium host cell. The invention relates to non-replicating linear integration cassette comprising at least one strong promoter operably linked to a polynucleotide encoding one or

more polypeptide(s) of interest, a 5' flanking polynucleotide segment upstream of the promoter of step (a), said segment comprising a first homologous region located in the 3' end of the segment, and a 3' flanking polynucleotide segment downstream of the polynucleotide of step (a), said segment comprising a second homologous region located in the 5' end of the segment. The consensus promoter has the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region. The consensus promoter is derived from one or more of the following genes: amyL, amyQ, amyE, amyM, cryliA, dagA, aprH, penP, sacB, spol, tac, xylA, and xylB. The invention relates to expression libraries for oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, membrane associated protein(s), or anti-microbial peptide(s).

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 3 OF 4 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:480640 HCAPLUS
DOCUMENT NUMBER: 135:75841
TITLE: Methods for producing a polypeptide in a Bacillus cell
INVENTOR(S): Widner, William; Sloma, Alan; Thomas, Michael D.
PATENT ASSIGNEE(S): Novozymes Biotech, Inc., USA
SOURCE: U.S., 54 pp., Cont.-in-part of U.S. 5,955,310.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6255076	B1	20010703	US 1999-258377	19990226
US 5955310	A	19990921	US 1998-31442	19980226
US 2003170876	A1	20030911	US 2001-834271	20010412

PRIORITY APPLN. INFO.: US 1998-31442 A2 19980226
US 1999-256377 B3 19990224

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a Bacillus host cell in a medium conducive for the production of the polypeptide, wherein the Bacillus cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a Bacillus host cell in a medium conducive for the production of the polypeptide, wherein the Bacillus cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:566201 HCAPLUS
DOCUMENT NUMBER: 131:180803
TITLE: Nucleic acid vectors for recombinant production of heterologous proteins in a Bacillus cell
INVENTOR(S): Widner, William; Sloma, Alan; Thomas, Michael D.

PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		
W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LU, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 5955310	A	19990921	US 1998-31442	19980226
AU 9929756	A1	19990915	AU 1999-29756	19990226
EP 1056873	A2	20001206	EP 1999-911012	19990226
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
JP 2002504379	T2	20020212	JP 2000-533574	19990226
US 2003170876	A1	20030911	US 2001-834271	20010412

PRIORITY APPLN. INFO.:
US 1998-31442 A 19980226
US 1999-256377 B3 19990224
WO 1999-US4360 W 19990226

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a Bacillus host cell in a medium conducive for the production of the polypeptide, wherein the Bacillus cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a Bacillus host cell in a medium conducive for the production of the polypeptide, wherein the Bacillus cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. Random promoters are created by placing promoters such as amyQ and amyL upstream of the cryIIIA promoter and its mRNA stabilizing sequence. Alternatively, "consensus" amyQ promoters are created with the cryIIIA mRNA stabilizing sequence, as well as tandom copies of a single promoter such as the short consensus amyQ dimer and trimer promoters. All of these approaches lead to significantly higher levels of SAVINASE gene expression (up to 620%) in Bacillus cells when compared to the levels obtained using single promoters such as amyQ and amyL.

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FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 09:47:21 ON 18 MAR 2004
L1 354831 S BACILLUS

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L2          107 S "TTGACA" AND "TATAAT"
L3           33 S L1 AND L2
L4        49123 S "AMYQ" OR "ALPHA-AMYLASE"
L5            0 S L1 (A)EMYLOLIQUEFACIENS
L6         5130 S L1 (A)AMYLOLIQUEFACIENS
L7            4 S L2 AND L6
L8            4 DUP REM L7 (0 DUPLICATES REMOVED)

=> s " and l3mRNA"
<------User Break------>

SEARCH ENDED BY USER

=> s " mRNA" and l3
L9           10 " MRNA" AND L3

=> dup rem l9
PROCESSING COMPLETED FOR L9
L10           6 DUP REM L9 (4 DUPLICATES REMOVED)

=> d 1-6 ibib ab

L10   ANSWER 1 OF 6  BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-02033  BIOTECHDS
TITLE:            Generating an expression library of polynucleotides by
                  introducing the linear integration cassette into the host
                  cell and selecting or screening for host cells that produce
                  the polypeptides of interest;
                     protein library screening using homologous recombination
AUTHOR:           BJORNVAD M E; JORGENSEN P L; HANSEN P K
PATENT ASSIGNEE:  NOVOZYMES AS
PATENT INFO:      WO 2003095658 20 Nov 2003
APPLICATION INFO: WO 2003-DK301 7 May 2003
PRIORITY INFO:    DK 2002-682 7 May 2002; DK 2002-682 7 May 2002
DOCUMENT TYPE:    Patent
LANGUAGE:         English
AB    DERWENT ABSTRACT:
      NOVELTY - Generating an expression library of polynucleotides integrated
      by homologous recombination into the genome of a competent Gram-positive
      bacterium host cell comprises: (a) providing a non-replicating linear
      integration cassette; and (b) introducing the linear integration cassette
      into the host cell and selecting or screening for host cells that produce
      the polypeptides of interest.
           DETAILED DESCRIPTION - Generating an expression library of
      polynucleotides integrated by homologous recombination into the genome of
      a competent Gram-positive bacterium host cell comprises: (a) providing a
      non-replicating linear integration cassette; and (b) introducing the
      linear integration cassette into the host cell and selecting or screening
      for host cells that produce the polypeptides of interest. The cassette
      comprises: (1) a polynucleotide encoding one or more polypeptides of
      interest; (2) a 5' flanking polynucleotide segment upstream of the
      polynucleotide of (1) and comprising a first homologous region located in
      the 3' end of the segment; and (3) a 3' flanking polynucleotide segment
      downstream of the polynucleotide of (1) and comprising a second
      homologous region located in the 5' end of the segment. The first and
      second homologous regions are at least 500, 1000 or 1500 bp, each of
      which has a sequence identity of at least 80, 85, 90 or 95-100% with a
      region of the host cell genom. INDEPENDENT CLAIMS are also included for
      the following: (1) a non-replicating linear Gram-positive host cell
      integration cassette; and (2) a method of producing a polypeptide of
      interest.
           BIOTECHNOLOGY - Preferred Method: Generating an expression library
      of polynucleotides integrated by homologous recombination into the genome
      of a competent Gram-positive bacterium host cell comprises the additional
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step carried out between steps (1) and (2) that comprises introducing the plasmid into an intermediate Escherichia coli host cell and propagating it by replication. The integration cassette comprises: (1) an **mRNA** processing/stabilizing sequence derived from cryIIIa-gene; (2) a terminator downstream of the polynucleotide encoding the polypeptides of interest; and (3) a multiple cloning site with at least one recognition site for a restriction nuclease. It further comprises a marker gene located between the 5' and 3' flanking segments and at least one promoter that is a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region, and/or which is derived from amyL, amyQ, amyM, cryIIIA, dagA, aprH, penP, sacB, spol, tac, xylA or xylB. The promoter is located between the flanking segments and is operably linked to the polynucleotide encoding one or more polypeptides of interest. Each of the 5' and 3' flanking polynucleotide segments comprises at least 500, 1000, 1500 or 2000 bp of non-homologous polynucleotides located in the 5' and 3' end of the 5' and 3' flanking segments, respectively. The promoter is one that results in that the host cells produce the polypeptides of interest in a yield of at least 10 mg/L. The polynucleotide comprises natural, synthetic or a library of shuffled or recombined homologs or variants of a gene or operon, provided by DNA breeding or DNA shuffling. The polypeptides of interest comprise enzymes, proteins or antimicrobial peptides. The enzymes are involved in the biosynthesis of hyaluronic acid. The Gram-positive host cell is **Bacillus** subtilis. The homologous region of the 5' and/or the 3' flanking segment is comprised in the yfmD-yfmC-yfmB-yfmA-pelB-yflS-citS region of the **Bacillus** subtilis genome or in the cryIIIa promoter. The non-replicating linear integration cassette is comprised in a plasmid and introduced into the host cell. The plasmid is capable of replicating in an Escherichia coli host cell but not in a **Bacillus** host cell. Producing a polypeptide of interest comprises culturing Gram-positive host cells comprising the integration cassette integrated into its genome, under conditions promoting expression of the polypeptide of interest. The method further comprises isolating and/or purifying the polypeptide of interest.

USE - The method is useful in generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell (claimed). (55 pages)

L10 ANSWER 2 OF 6 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-00390 BIOTECHDS
TITLE: Creating a library of artificial promoters comprises mixing oligonucleotides in a polymerase chain reaction with an insertion DNA cassette to obtain a library of double-stranded amplified products comprising artificial promoters; artificial protein library construction and vector expression in host cell for use in gene expression level determination
AUTHOR: SOUCAILLE P
PATENT ASSIGNEE: GENENCOR INT INC
PATENT INFO: WO 2003089605 30 Oct 2003
APPLICATION INFO: WO 2003-US12045 18 Apr 2003
PRIORITY INFO: US 2002-374627 22 Apr 2002; US 2002-374627 22 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-854112 [79]
AB DERWENT ABSTRACT:

NOVELTY - Creating a library of artificial promoters comprises mixing a first oligonucleotide and a second oligonucleotide in an amplification reaction with an insertion DNA cassette to obtain a library of double-stranded amplified products comprising artificial promoters.

DETAILED DESCRIPTION - The above method comprises: (a) obtaining an insertion DNA cassette comprising a first recombinase site, a second recombinase site and a selective marker gene located between the first

and the second recombinase sites; (b) obtaining a first oligonucleotide comprising a first nucleic acid fragment homologous to an upstream region of a chromosomal gene of interest, and a second nucleic acid fragment homologous to a 5' end of the insertion DNA cassette; (c) obtaining a second oligonucleotide comprising (i) a third nucleic acid fragment homologous to a 3' end of the insertion DNA cassette, (ii) a precursor promoter comprising a -35 consensus region (-35 to -30), a linker sequence and a -10 consensus region (-2 to -7), where the linker sequence comprises 4-20 nucleotides and is flanked by the -35 region and the -10 region, where the precursor promoter has been modified to include at least one modified nucleotide position of the promoter and where the -35 region and the -10 region each include 4-6 conserved nucleotides of the promoter, and (iii) a fourth nucleic acid fragment homologous to a downstream region of the transcription start site of the promoter; and (d) mixing the first oligonucleotide and the second oligonucleotide in an amplification reaction with the insertion DNA cassette to obtain a library of double-stranded amplified products comprising artificial promoters. INDEPENDENT CLAIMS are also included for the following: (1) an artificial promoter library comprising a mixture of double-stranded polynucleotides which include, in sequential order: a nucleic acid fragment homologous to an upstream region of a chromosomal gene of interest; a first recombinase site; a nucleic acid sequence encoding an antimicrobial resistance gene; a second recombinase gene; 2 consensus regions of a promoter and a linker sequence, where the first consensus region comprises the -35 region and the second region comprises the -10 region cited above; and a nucleic acid fragment homologous to the downstream region of the +1 transcription start site of the promoter; (2) methods of modifying a promoter in selected host cells; (3) a method of creating a library of bacterial cells having a range of expression levels of a chromosomal gene of interest; and (4) transformed bacterial cells selected from the method in (3).

BIOTECHNOLOGY - Preferred Method: Creating a library of artificial promoters further comprises purifying the amplified products. The amplification step is a polymerase chain reaction (PCR) step. The -35 region of the precursor promoter is selected from **TTGACA**, TTGCTA, TTGCTT, TTGATA, TTGACT, TTTACA and TTCAAA. It comprises a modification to the -30 residue of the promoter. The -10 region is selected from TAAGAT, **TATAAT**, AATAAT, TATACT, GATACT, TACGAT, TATGTT and GACAAT. Preferably, the -35 region is **TTGACA** and the -10 region is **TATAAT** or AATAAT. The linker sequence comprises 16-18 nucleotides. The precursor promoter is obtained from a promoter selected from Ptrc, PD/E20, PH207, PN25, PG25, PJ5, PA1, PA2, PA3, Plac, PGI, PlacUV5, PCON, and Pbls. Each of the precursor promoters comprises a sequence fully defined in the specification. The library of artificial promoters includes 3 sequences of 60 bp each fully defined in the specification. The precursor promoter and the chromosomal gene of interest are homologous or heterologous. The method further comprises modifying the ribosome binding site, including: (a) obtaining a third oligonucleotide comprising a fifth nucleic acid fragment homologous to the 5' end of the chromosomal gene of interest; a modified ribosome binding site of the gene of interest, the binding site includes at least one modified nucleotide; and a sixth nucleic acid fragment homologous to a downstream region of the -10 region of the second oligonucleotide; and (b) mixing the PCR products with the third oligonucleotide and the first oligonucleotide in a PCR reaction to obtain PCR products comprising artificial promoters with modified ribosome binding sites. The ribosome binding site from the precursor promoter is selected from any of the 27 nucleotide sequences (e.g. AGGAAA, AGAAAA or AGAAGA) fully defined in the specification. The method further comprises inserting a stabilizing **mRNA** sequence between the modified ribosome binding site and a transcription initiation site of the third oligonucleotide, and altering the start codon of the gene of interest in the third oligonucleotide. Alternatively, the method comprises: (a) obtaining a third oligonucleotide comprising a fifth nucleic acid fragment homologous to

the 5' end of the chromosomal gene of interest; a start codon of the gene of interest, where the start codon is degenerated and includes at least one modification; and a sixth nucleic acid fragment homologous to a downstream region of the -10 region of the second oligonucleotide; and (b) mixing the PCR products with the third oligonucleotide and the first oligonucleotide in a PCR reaction to obtain PCR products comprising artificial promoters with modified start codons. It also comprises inserting a stabilizing **mRNA** sequence between the -10 box of the artificial promoter and a transcription initiation site of the third oligonucleotide. Modifying a promoter in selected host cells comprises obtaining a library of PCR products comprising artificial promoters cited above; transforming bacterial host cells with the PCR library, where the PCR products comprising the artificial promoters are integrated into the bacterial host cells by homologous recombination; growing the transformed bacterial cells; and selecting the transformed bacterial cells comprising the artificial promoters. The bacterial host cell is selected from Escherichia coli, Pantoea sp. and **Bacillus** sp.. Creating a library of bacterial cells having a range of expression levels of a chromosomal gene of interest comprises obtaining a library of PCR products comprising artificial promoters cited above; transforming bacterial host cells with the PCR products, where the PCR products comprising the artificial promoters are integrated into bacterial host cells by homologous recombination to produce transformed bacterial cells; growing the transformed bacteria cells; and obtaining a library of transformed bacterial cells where the library exhibits a range of expression levels of a chromosomal gene of interest. The method further comprises selecting transformed bacterial cells from the library. The selected transformed bacterial cells have a low or high level of expression of the gene of interest. The method also comprises excising the selective marker gene from the transformed bacterial cells. Preferred Promoter Library: The double-stranded polynucleotides further include a modified ribosome binding site of the promoter, a modified start codon or a stabilizing **mRNA** nucleic acid sequence, where the binding site, start codon or **mRNA** sequence is located between the -10 region and the nucleic acid sequence homologous to the downstream region of the +1 transcription start site. The -35 region includes a substitution in one nucleotide position with the remaining nucleotide positions conserved. The promoter library further includes a substitution in one nucleotide position of the -10 region with the remaining nucleotide positions conserved.

USE - The method is useful in creating a library of bacterial clones with varying levels of gene expression. The method is used in developing a quick and efficient means of determining the optimum expression level of a gene in a metabolic pathway which, in turn, results in an optimization of strain performance for a desired product.

ADVANTAGE - A direct advantage of the method is that a bacterial clone may be selected based on the expression level obtained from the DNA libraries and then be ready for use in a fermentation process where cell viability is not negatively affected by expression of the gene of interest. (44 pages)

L10 ANSWER 3 OF 6 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: WIDNER W; SLOMA A; THOMAS M D
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003
APPLICATION INFO: US 2001-834271 12 Apr 2001

PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]
AB DERWENT ABSTRACT:
NOVELTY - Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a **Bacillus** cell comprising a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an **mRNA** processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a **Bacillus** host cell by introducing into a **Bacillus** cell the nucleic acid construct cited above; (3) a method for producing a selectable marker-free mutant of a **Bacillus** cell by deleting a selectable marker gene of the **Bacillus** cell; and (4) a selectable marker-free mutant of a **Bacillus** cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an **mRNA** processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter comprises two or more bacterial promoter sequences, which are obtained from one or more **Bacillus** genes. The tandem promoter comprises the amyQ promoter, a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** from the -10 region, the amyL promoter, and/or the cryIIIA promoter. The tandem promoter comprises two copies of the amyQ, amyl or cryIIIA promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the **Bacillus** cell. The **mRNA** processing/stabilizing sequence is the cryIIIA or SP82 **mRNA** processing/stabilizing sequence, which generates **mRNA** transcripts essentially of the same size. The **Bacillus** cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the **Bacillus** cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the **Bacillus** cell. The **Bacillus** host cell is **Bacillus** alkalophilus, **Bacillus** amyloliquefaciens, **Bacillus** brevis, **Bacillus** brevis, **Bacillus** circulans, **Bacillus** clausii, **Bacillus** coagulans, **Bacillus** firmus, **Bacillus** lautus, **Bacillus** lentus, **Bacillus** licheniformis, **Bacillus** megaterium, **Bacillus** pumilus, **Bacillus** sterothermophilus,

Bacillus subtilis, or **Bacillus** thuringiensis. This method alternatively comprises cultivating a **Bacillus** cell in a medium conducive for the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an **mRNA** processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a **Bacillus** promoter. Preferred Cell: The **Bacillus** cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a **Bacillus** cell, and for producing a selectable marker-free mutant of a **Bacillus** cell.

EXAMPLE - No relevant example given.(57 pages)

L10 ANSWER 4 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2

ACCESSION NUMBER: 2001:378829 BIOSIS
DOCUMENT NUMBER: PREV200100378829
TITLE: Methods for producing a polypeptide in a **Bacillus** cell.
AUTHOR(S): Widner, William [Inventor, Reprint author]; Sloma, Alan [Inventor]; Thomas, Michael D. [Inventor]
CORPORATE SOURCE: Davis, CA, USA
ASSIGNEE: Novozymes Biotech, Inc., Davis, CA, USA
PATENT INFORMATION: US 6255076 July 03, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (July 3, 2001) Vol. 1248, No. 1. e-file. CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Aug 2001
Last Updated on STN: 19 Feb 2002

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and alternatively also (ii) an **mRNA** processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an **mRNA** processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium.

L10 ANSWER 5 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:566201 HCAPLUS
DOCUMENT NUMBER: 131:180803
TITLE: Nucleic acid vectors for recombinant production of

heterologous proteins in a **Bacillus** cell
INVENTOR(S): Widner, William; Sloma, Alan; Thomas, Michael D.
PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		
W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LU, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 5955310	A	19990921	US 1998-31442	19980226
AU 9929756	A1	19990915	AU 1999-29756	19990226
EP 1056873	A2	20001206	EP 1999-911012	19990226
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
JP 2002504379	T2	20020212	JP 2000-533574	19990226
US 2003170876	A1	20030911	US 2001-834271	20010412

PRIORITY APPLN. INFO.:
US 1998-31442 A 19980226
US 1999-256377 B3 19990224
WO 1999-US4360 W 19990226

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and alternatively also (ii) an **mRNA** processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an **mRNA** processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. Random promoters are created by placing promoters such as amyQ and amyL upstream of the cryIIIA promoter and its **mRNA** stabilizing sequence. Alternatively, "consensus" amyQ promoters are created with the cryIIIA **mRNA** stabilizing sequence, as well as tandom copies of a single promoter such as the short consensus amyQ dimer and trimer promoters. All of these approaches lead to significantly higher levels of SAVINASE gene expression (up to 620%) in **Bacillus** cells when compared to the levels obtained using single promoters such as amyQ and amyL.

L10 ANSWER 6 OF 6 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 83012205 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6181373
TITLE: Nucleotide sequences that signal the initiation of transcription and translation in **Bacillus**

subtilis.
AUTHOR: Moran C P Jr; Lang N; LeGrice S F; Lee G; Stephens M; Sonenshein A L; Pero J; Losick R
CONTRACT NUMBER: GM18568 (NIGMS) GM19168 (NIGMS)
SOURCE: Molecular & general genetics : MGG, (1982) 186 (3) 339-46. Journal code: 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-J01550; GENBANK-J01552; GENBANK-J01553
ENTRY MONTH: 198212
ENTRY DATE: Entered STN: 19900317
Last Updated on STN: 19980206
Entered Medline: 19821202

AB We have determined the nucleotide sequence of two **Bacillus** subtilis promoters (veg and tms) that are utilized by the principal form of B. subtilis RNA polymerase found in vegetative cells (sigma 55-RNA polymerase) and have compared our sequences to those of several previously reported **Bacillus** promoters. Hexanucleotide sequences centered approximately 35 (the "--35" region) and 10 (the "--10" region) base pairs upstream from the veg and tms transcription starting points (and separated by 17 base pairs) corresponded closely to the consensus hexanucleotides (**TTGACA** and **TATAAT**) attributed to Escherichia coli promoters. Conformity to the preferred --35 and --10 sequences may not be sufficient to promote efficient utilization by B. subtilis RNA polymerase, however, since three promoters (veg, tms and E. coli tac) that conform to these sequences and that are utilized efficiently by E. coli RNA polymerase were used with highly varied efficiencies by B. subtilis RNA polymerase. We have also analyzed **mRNA** sequences in DNA located downstream from eight B. subtilis chromosomal and phage promoters for nucleotide sequences that might signal the initiation of translation. In accordance with the rules of McLaughlin, Murray and Rabinowitz (1981), we observe **mRNA** nucleotide sequences with extensive complementarity to the 3' terminal region of B. subtilis 16S rRNA, followed by an initiation codon and an open reading frame.

```
=> s "cryIIIA" or "SP82"
L11        788 "CRYIIIA" OR "SP82"

=> d his

     (FILE 'HOME' ENTERED AT 09:46:39 ON 18 MAR 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 09:47:21 ON 18 MAR 2004
L1        354831 S BACILLUS
L2           107 S "TTGACA" AND "TATAAT"
L3            33 S L1 AND L2
L4         49123 S "AMYQ" OR "ALPHA-AMYLASE"
L5             0 S L1 (A)EMYLOLIQUEFACIENS
L6          5130 S L1 (A)AMYLOLIQUEFACIENS
L7             4 S L2 AND L6
L8             4 DUP REM L7 (0 DUPLICATES REMOVED)
L9            10 S " MRNA" AND L3
L10            6 DUP REM L9 (4 DUPLICATES REMOVED)
L11          788 S "CRYIIIA" OR "SP82"

=> s l3 and l11
L12            4 L3 AND L11

=> dup rem l12
```

PROCESSING COMPLETED FOR L12
L13 3 DUP REM L12 (1 DUPLICATE REMOVED)

=> d 1-3 ibib ab

L13 ANSWER 1 OF 3 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-02033 BIOTECHDS
TITLE: Generating an expression library of polynucleotides by introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest;
protein library screening using homologous recombination
AUTHOR: BJORNVAD M E; JORGENSEN P L; HANSEN P K
PATENT ASSIGNEE: NOVOZYMES AS
PATENT INFO: WO 2003095658 20 Nov 2003
APPLICATION INFO: WO 2003-DK301 7 May 2003
PRIORITY INFO: DK 2002-682 7 May 2002; DK 2002-682 7 May 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
AB DERWENT ABSTRACT:

NOVELTY - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest.

DETAILED DESCRIPTION - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest. The cassette comprises: (1) a polynucleotide encoding one or more polypeptides of interest; (2) a 5' flanking polynucleotide segment upstream of the polynucleotide of (1) and comprising a first homologous region located in the 3' end of the segment; and (3) a 3' flanking polynucleotide segment downstream of the polynucleotide of (1) and comprising a second homologous region located in the 5' end of the segment. The first and second homologous regions are at least 500, 1000 or 1500 bp, each of which has a sequence identity of at least 80, 85, 90 or 95-100% with a region of the host cell genom. INDEPENDENT CLAIMS are also included for the following: (1) a non-replicating linear Gram-positive host cell integration cassette; and (2) a method of producing a polypeptide of interest.

BIOTECHNOLOGY - Preferred Method: Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises the additional step carried out between steps (1) and (2) that comprises introducing the plasmid into an intermediate Escherichia coli host cell and propagating it by replication. The integration cassette comprises: (1) an mRNA processing/stabilizing sequence derived from **cryIIIa**-gene; (2) a terminator downstream of the polynucleotide encoding the polypeptides of interest; and (3) a multiple cloning site with at least one recognition site for a restriction nuclease. It further comprises a marker gene located between the 5' and 3' flanking segments and at least one promoter that is a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region, and/or which is derived from amyL, amyQ, amyM, **cryIIIA**, dagA, aprH, penP, sacB, spoI, tac, xylA or xylB. The promoter is located between the flanking segments and is operably linked to the polynucleotide encoding one or more polypeptides of interest. Each of the 5' and 3' flanking polynucleotide segments comprises at least 500, 1000, 1500 or 2000 bp of non-homologous polynucleotides located in the 5' and 3' end of the 5' and 3' flanking segments, respectively. The promoter is

one that results in that the host cells produce the polypeptides of interest in a yield of at least 10 mg/L. The polynucleotide comprises natural, synthetic or a library of shuffled or recombined homologs or variants of a gene or operon, provided by DNA breeding or DNA shuffling. The polypeptides of interest comprise enzymes, proteins or antimicrobial peptides. The enzymes are involved in the biosynthesis of hyaluronic acid. The Gram-positive host cell is **Bacillus** subtilis. The homologous region of the 5' and/or the 3' flanking segment is comprised in the yfmD-yfmC-yfmB-yfmA-pelB-yflS-citS region of the **Bacillus** subtilis genome or in the **cryIIIa** promoter. The non-replicating linear integration cassette is comprised in a plasmid and introduced into the host cell. The plasmid is capable of replicating in an Escherichia coli host cell but not in a **Bacillus** host cell. Producing a polypeptide of interest comprises culturing Gram-positive host cells comprising the integration cassette integrated into its genome, under conditions promoting expression of the polypeptide of interest. The method further comprises isolating and/or purifying the polypeptide of interest.

USE - The method is useful in generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell (claimed). (55 pages)

L13 ANSWER 2 OF 3 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: WIDNER W; SLOMA A; THOMAS M D
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003
APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]
AB DERWENT ABSTRACT:

NOVELTY - Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a **Bacillus** cell comprising a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a **Bacillus** host cell by introducing into a **Bacillus** cell the nucleic acid construct cited above; (3) a method for producing a selectable marker-free mutant of a **Bacillus** cell by deleting a selectable marker gene of the **Bacillus** cell; and (4) a selectable marker-free mutant of a **Bacillus** cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter

comprises two or more bacterial promoter sequences, which are obtained from one or more **Bacillus** genes. The tandem promoter comprises the amyQ promoter, a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** from the -10 region, the amyL promoter, and/or the **cryIIIA** promoter. The tandem promoter comprises two copies of the amyQ, amyl or **cryIIIA** promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the **Bacillus** cell. The mRNA processing/stabilizing sequence is the **cryIIIA** or **SP82** mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The **Bacillus** cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the **Bacillus** cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the **Bacillus** cell. The **Bacillus** host cell is **Bacillus** alkalophilus, **Bacillus** amyloliquefaciens, **Bacillus** brevis, **Bacillus** brevis, **Bacillus** circulans, **Bacillus** clausii, **Bacillus** coagulans, **Bacillus** firmus, **Bacillus** lautus, **Bacillus** lentus, **Bacillus** licheniformis, **Bacillus** megaterium, **Bacillus** pumilus, **Bacillus** sterothermophilus, **Bacillus** subtilis, or **Bacillus** thuringiensis. This method alternatively comprises cultivating a **Bacillus** cell in a medium conducive for the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a **Bacillus** promoter. Preferred Cell: The **Bacillus** cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a **Bacillus** cell, and for producing a selectable marker-free mutant of a **Bacillus** cell.

EXAMPLE - No relevant example given.(57 pages)

L13 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1999:566201 HCAPLUS

DOCUMENT NUMBER: 131:180803

TITLE: Nucleic acid vectors for recombinant production of heterologous proteins in a **Bacillus** cell

INVENTOR(S): Widner, William; Sloma, Alan; Thomas, Michael D.

PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA

SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		
W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LU, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 5955310	A	19990921	US 1998-31442	19980226
AU 9929756	A1	19990915	AU 1999-29756	19990226
EP 1056873	A2	20001206	EP 1999-911012	19990226
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
JP 2002504379	T2	20020212	JP 2000-533574	19990226
US 2003170876	A1	20030911	US 2001-834271	20010412

PRIORITY APPLN. INFO.:

US 1998-31442	A	19980226
US 1999-256377	B3	19990224
WO 1999-US4360	W	19990226

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. Random promoters are created by placing promoters such as amyQ and amyL upstream of the **cryIIIA** promoter and its mRNA stabilizing sequence. Alternatively, "consensus" amyQ promoters are created with the **cryIIIA** mRNA stabilizing sequence, as well as tandom copies of a single promoter such as the short consensus amyQ dimer and trimer promoters. All of these approaches lead to significantly higher levels of SAVINASE gene expression (up to 620%) in **Bacillus** cells when compared to the levels obtained using single promoters such as amyQ and amyL.

```
=> e widner w/au
E1          32     WIDNER T E/AU
E2           2     WIDNER THOMAS E/AU
E3          30 --> WIDNER W/AU
E4           7     WIDNER W E/AU
E5          41     WIDNER W R/AU
E6          13     WIDNER WILLIAM/AU
E7          18     WIDNER WILLIAM R/AU
E8           1     WIDNER WILLIAM ROY/AU
E9           1     WIDNER WM R/AU
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E10           2      WIDNES J/AU
E11           2      WIDNES J A/AU
E12           2      WIDNES V/AU

=> s e3-e8
L14         110 ("WIDNER W"/AU OR "WIDNER W E"/AU OR "WIDNER W R"/AU OR "WIDNER
                WILLIAM"/AU OR "WIDNER WILLIAM R"/AU OR "WIDNER WILLIAM ROY"/AU)


=> e sloma a/au
E1            3      SLOM T J/AU
E2            2      SLOM TREVOR J/AU
E3          114 --> SLOMA A/AU
E4           15      SLOMA A P/AU
E5           51      SLOMA ALAN/AU
E6           12      SLOMA ALAN P/AU
E7            1      SLOMA ALAN PAUL/AU
E8            1      SLOMA D/AU
E9            1      SLOMA D R/AU
E10           6      SLOMA E/AU
E11           2      SLOMA E J/AU
E12           2      SLOMA J/AU

=> s e3-e7
L15         193 ("SLOMA A"/AU OR "SLOMA A P"/AU OR "SLOMA ALAN"/AU OR "SLOMA
                ALAN P"/AU OR "SLOMA ALAN PAUL"/AU)

=> e thomas m d/au
E1            4      THOMAS M C C/AU
E2           12      THOMAS M CARMEN/AU
E3          387 --> THOMAS M D/AU
E4           93      THOMAS M D A/AU
E5            4      THOMAS M D H/AU
E6            3      THOMAS M D JR/AU
E7            2      THOMAS M D O/AU
E8           13      THOMAS M D R/AU
E9          331      THOMAS M E/AU
E10          33      THOMAS M E A/AU
E11          32      THOMAS M E M/AU
E12           2      THOMAS M ELIZABETH/AU

=> s e3
L16         387 "THOMAS M D"/AU

=> d his

     (FILE 'HOME' ENTERED AT 09:46:39 ON 18 MAR 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 09:47:21 ON 18 MAR 2004
L1       354831 S BACILLUS
L2          107 S "TTGACA" AND "TATAAT"
L3           33 S L1 AND L2
L4        49123 S "AMYQ" OR "ALPHA-AMYLASE"
L5            0 S L1 (A)EMYLOLIQUEFACIENS
L6         5130 S L1 (A)AMYLOLIQUEFACIENS
L7            4 S L2 AND L6
L8            4 DUP REM L7 (0 DUPLICATES REMOVED)
L9           10 S " MRNA" AND L3
L10           6 DUP REM L9 (4 DUPLICATES REMOVED)
L11         788 S "CRYIIIA" OR "SP82"
L12           4 S L3 AND L11
L13           3 DUP REM L12 (1 DUPLICATE REMOVED)
                E WIDNER W/AU
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L14          110 S E3-E8
                 E SLOMA A/AU
L15          193 S E3-E7
                 E THOMAS M D/AU
L16          387 S E3

=> s l14 or l15 or l16
L17          652 L14 OR L15 OR L16

=> s l3 and l17
L18            4 L3 AND L17

=> dup rem l18
PROCESSING COMPLETED FOR L18
L19            3 DUP REM L18 (1 DUPLICATE REMOVED)

=> d 1-3 ibib ab

L19   ANSWER 1 OF 3  BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-04169  BIOTECHDS
TITLE:            Producing a polypeptide comprises cultivating a
                  Bacillus cell in a medium conducive to the production
                  of the polypeptide, where the Bacillus cell
                  comprises a nucleic acid construct comprising a tandem
                  promoter;
                     involving vector-mediated gene transfer and expression in
                     host cell for use as a selectable marker
AUTHOR:           WIDNER W; SLOMA A; THOMAS M D
PATENT ASSIGNEE:  NOVOZYMES BIOTECH INC
PATENT INFO:      US 2003170876 11 Sep 2003
APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO:    US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     WPI: 2003-898275 [82]
AB    DERWENT ABSTRACT:
      NOVELTY - Producing a polypeptide comprises cultivating a
      Bacillus cell in a medium conducive to the production of the
      polypeptide, where the Bacillus cell comprises a nucleic acid
      construct comprising a tandem promoter in which each promoter sequence of
      the tandem promoter is operably linked to a nucleic acid sequence
      encoding the polypeptide, and isolating the polypeptide from the
      cultivation medium.
           DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the
      following: (1) a Bacillus cell comprising a nucleic acid
      construct comprising a tandem promoter in which each promoter sequence of
      the tandem promoter is operably linked to a single copy of a nucleic acid
      sequence encoding a polypeptide, and optionally an mRNA
      processing/stabilizing sequence located downstream of the tandem promoter
      and upstream of the nucleic acid sequence encoding the polypeptide; (2) a
      method for obtaining a Bacillus host cell by introducing into a
      Bacillus cell the nucleic acid construct cited above; (3) a
      method for producing a selectable marker-free mutant of a
      Bacillus cell by deleting a selectable marker gene of the
      Bacillus cell; and (4) a selectable marker-free mutant of a
      Bacillus cell.
           BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the
      nucleic acid construct further comprises an mRNA processing/stabilizing
      sequence located downstream of the tandem promoter and upstream of the
      nucleic acid sequence encoding the polypeptide. The tandem promoter
      comprises two or more bacterial promoter sequences, which are obtained
      from one or more Bacillus genes. The tandem promoter comprises
      the amyQ promoter, a consensus promoter having the sequence
      TTGACA for the -35 region and TATAAT from the -10
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region, the amyL promoter, and/or the cryIIIA promoter. The tandem promoter comprises two copies of the amyQ, amyl or cryIIIA promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the **Bacillus** cell. The mRNA processing/stabilizing sequence is the cryIIIA or SP82 mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The **Bacillus** cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the **Bacillus** cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the **Bacillus** cell. The **Bacillus** host cell is **Bacillus** alkalophilus, **Bacillus** amyloliquefaciens, **Bacillus** brevis, **Bacillus** brevis, **Bacillus** circulans, **Bacillus** clausii, **Bacillus** coagulans, **Bacillus** firmus, **Bacillus** lautus, **Bacillus** lentus, **Bacillus** licheniformis, **Bacillus** megaterium, **Bacillus** pumilus, **Bacillus** sterothermophilus, **Bacillus** subtilis, or **Bacillus** thuringiensis. This method alternatively comprises cultivating a **Bacillus** cell in a medium conducive for the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a **Bacillus** promoter. Preferred Cell: The **Bacillus** cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a **Bacillus** cell, and for producing a selectable marker-free mutant of a **Bacillus** cell.

EXAMPLE - No relevant example given.(57 pages)

L19 ANSWER 2 OF 3 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1

ACCESSION NUMBER: 2001:378829 BIOSIS
DOCUMENT NUMBER: PREV200100378829
TITLE: Methods for producing a polypeptide in a **Bacillus** cell.
AUTHOR(S): **Widner, William** [Inventor, Reprint author]; **Sloma, Alan** [Inventor]; Thomas, Michael D. [Inventor]
CORPORATE SOURCE: Davis, CA, USA
ASSIGNEE: Novozymes Biotech, Inc., Davis, CA, USA
PATENT INFORMATION: US 6255076 July 03, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (July 3, 2001) Vol. 1248, No. 1. e-file.

CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Aug 2001
Last Updated on STN: 19 Feb 2002

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium.

L19 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:566201 HCAPLUS
DOCUMENT NUMBER: 131:180803
TITLE: Nucleic acid vectors for recombinant production of heterologous proteins in a **Bacillus** cell
INVENTOR(S): **Widner, William; Sloma, Alan;** Thomas, Michael D.
PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		
W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LU, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 5955310	A	19990921	US 1998-31442	19980226
AU 9929756	A1	19990915	AU 1999-29756	19990226
EP 1056873	A2	20001206	EP 1999-911012	19990226
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
JP 2002504379	T2	20020212	JP 2000-533574	19990226
US 2003170876	A1	20030911	US 2001-834271	20010412

PRIORITY APPLN. INFO.:
US 1998-31442 A 19980226
US 1999-256377 B3 19990224
WO 1999-US4360 W 19990226

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium

conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. Random promoters are created by placing promoters such as amyQ and amyL upstream of the cryIIIA promoter and its mRNA stabilizing sequence. Alternatively, "consensus" amyQ promoters are created with the cryIIIA mRNA stabilizing sequence, as well as tandom copies of a single promoter such as the short consensus amyQ dimer and trimer promoters. All of these approaches lead to significantly higher levels of SAVINASE gene expression (up to 620%) in **Bacillus** cells when compared to the levels obtained using single promoters such as amyQ and amyL.

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=> d his

     (FILE 'HOME' ENTERED AT 09:46:39 ON 18 MAR 2004)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 09:47:21 ON 18 MAR 2004
L1        354831 S BACILLUS
L2           107 S "TTGACA" AND "TATAAT"
L3            33 S L1 AND L2
L4         49123 S "AMYQ" OR "ALPHA-AMYLASE"
L5             0 S L1 (A)EMYLOLIQUEFACIENS
L6          5130 S L1 (A)AMYLOLIQUEFACIENS
L7             4 S L2 AND L6
L8             4 DUP REM L7 (0 DUPLICATES REMOVED)
L9            10 S " MRNA" AND L3
L10            6 DUP REM L9 (4 DUPLICATES REMOVED)
L11          788 S "CRYIIIA" OR "SP82"
L12            4 S L3 AND L11
L13            3 DUP REM L12 (1 DUPLICATE REMOVED)
                 E WIDNER W/AU
L14          110 S E3-E8
                 E SLOMA A/AU
L15          193 S E3-E7
                 E THOMAS M D/AU
L16          387 S E3
L17          652 S L14 OR L15 OR L16
L18            4 S L3 AND L17
L19            3 DUP REM L18 (1 DUPLICATE REMOVED)
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	Issue Date	Pages	Document ID	Title
1	20031002	22	US 20030186380 A1	Methods for producing secreted polypeptides having L-asparaginase activity
2	20030918	142	US 20030175902 A1	Methods for producing hyaluronan in a recombinant host cell
3	20030911	57	US 20030170876 A1	Methods for producing a polypeptide in a bacillus cell
4	20030807	12	US 20030148461 A1	Mutant aprE promoter
5	20030422	59	US 6551813 B1	Nutrient medium for bacterial strains which overproduce riboflavin
6	20030121	10	US 6509185 B1	Mutant aprE promotor
7	20020423	16	US 6376235 B1	IVI-2, IVI-3 and IVI-4 loci of Enterococcus faecalis polynucleotide, polypeptides and method of use therefor
8	20010703	54	US 6255076 B1	Methods for producing a polypeptide in a Bacillus cell
9	20000229	94	US 6030807 A	Highly regulable promoter for heterologous gene expression

	Issue Date	Pages	Document ID	Title
10	19930907	16	US 5243039 A	Bacillus MGA3 aspartokinase II gene
11	19921215	20	US 5171673 A	Expression of heterologous DNA using the bacillus coagulans amylase gene
12	19921006	16	US 5153120 A	Process for the production of .gamma.-glutamyl transpeptidase
13	19910219	10	US 4994380 A	Process for expressing genes by Bacillus brevis

	Issue Date	Pages	Document ID	Title
1	20030911	57	US 20030170876 A1	Methods for producing a polypeptide in a bacillus cell
2	20010703	54	US 6255076 B1	Methods for producing a polypeptide in a Bacillus cell
3	20000229	94	US 6030807 A	Highly regulable promoter for heterologous gene expression

	Issue Date	Pages	Document ID	Title
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2	20030911	57	US 20030170876 A1	Methods for producing a polypeptide in a bacillus cell
3	20010703	54	US 6255076 B1	Methods for producing a polypeptide in a Bacillus cell
4	19990921	39	US 5955310 A	Methods for producing a polypeptide in a bacillus cell
5	19970722	45	US 5650326 A	Promoter element and signal peptide of a gene encoding a Bacillus alkaline protease and vectors comprising same
6	19970422	45	US 5622850 A	Recombinant methods for the production of a bacillus alkaline protease

	Issue Date	Pages	Document ID	Title
7	19970422	45	US 5622841 A	Method for the production of heterologous polypeptides using a promoter element and signal peptide of a bacillus gene encoding an alkaline protease
8	19970415	45	US 5621089 A	Nucleic acid constructs for the production of a Bacillus alkaline protease

	Issue Date	Pages	Document ID	Title
1	20031002	22	US 20030186380 A1	Methods for producing secreted polypeptides having L-asparaginase activity
2	20030918	142	US 20030175902 A1	Methods for producing hyaluronan in a recombinant host cell
3	20030911	57	US 20030170876 A1	Methods for producing a polypeptide in a bacillus cell
4	20030422	59	US 6551813 B1	Nutrient medium for bacterial strains which overproduce riboflavin
5	20010703	54	US 6255076 B1	Methods for producing a polypeptide in a Bacillus cell
6	19921215	20	US 5171673 A	Expression of heterologous DNA using the bacillus coagulans amylase gene

	L #	Hits	Search Text
1	L1	30779	bacillus
2	L2	385	"TTGACA" or "TATAAT"
3	L3	1575	"AMY Q" or "amyloliquefaciens"
4	L4	0	l2 same "l6"
5	L5	13	l1 same l2
6	L6	50664	"mRNA"
7	L7	3	l5 same l6
8	L8	325	"cryIIIa" or "sp82"
9	L9	8	l3 same l8
10	L10	350158	SLOMA or THOMAS or WIDNER
11	L11	6	l5 and l10